Start up of the FACSCalibur flow cytometer

This is not intended to be a substitute for the manual and does not take the place of training. Rather, it is simply to remind you of the basics. If you need additional information, refer to the FACSCalibur and CellQuest Pro software manuals. You can also contact Wolf at 301-435-7272 or lindwasw@mail.nih.gov. Many thanks to Jen Gillette for writing a draft of these protocols.

Turn on the cytometer:

The power switch is the green button on the right side of the cytometer. The first thing you will do when you approach the machine is to press this button to the on (lit) position. The carousel should come to life and the control panel for the carousel should light up.

Turn on the computer:

It's important that the computer be turned on *after* the cytometer. If it's already on, you can Restart after you switch on the cytometer. Once the computer starts up, login as FACSCalibur Users and enter the password FACS (all caps please). Open the CellQuest Pro program. Choose "Connect to Cytometer" from the ACQUIRE menu.

Check Sheath and Waste tanks:

Open the drawer of the cytometer and check the Sheath and Waste tanks. Make sure the system is not pressurized (the toggle switch to the left of the tanks should be set to VENT-TANK CHANGE). The Sheath tank should be roughly ¾ full and the Waste shouldn't be too full.

To fill the Sheath tank, remove the black plate (push back and pull up). Unscrew cap and remove plug (put on towel to prevent dripping). Add FACS Flow (or PBS), which is in the 2L bottle on top of machine, or in the boxes below the machine. Screw the cap back on and replace the plate. When properly oriented, a tab on the cover plate will depress a black button.

If the Waste tank is more than ½ full, pour out the contents into the sink. Add 100 - 200 ml of neat bleach (Clorox) to the tank and put it back in the cytometer.

Flip the toggle switch to PRESSURIZE-RUN.

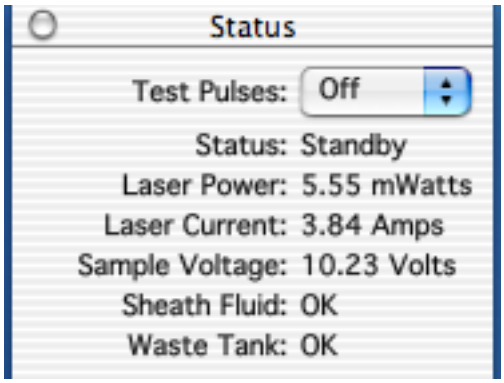
Check fluidics and lasers:

Place a tube with ~3 ml dH_2O into the SIP (sample injection port - looks like a little metal drinking straw). Press PRIME. Fluid should begin flowing. Wait a couple of seconds and then press RUN. The RUN light should turn green, indicating that the system is sufficiently pressurized to get the fluid moving. If it's yellow or orange it is in STANDBY mode. If it doesn't turn green after a few seconds, make sure the tanks are closed properly and check the dH_2O tube for cracks.

Do a visual check for bubbles. Bubble may be in the saline filter line. To get bubble out of the line, press the metal clamps to release the tube. When you push the end of the tube into a waste container, it will flush the tubing. There is also a stopper on the tubing that can be released to start the flow from the Sheath tank through the lines to remove bubbles.

Choose "Status" from the CYTOMETER menu. Once the lasers are warmed up the Status window should say "Ready". It takes about 5 minutes from power up for the lasers to be ready. Press STANDBY – Sample Voltage should change to 10.23 volts. This means the system is ready to go. See Fig. 1 for an example.

Figure 1: Standby mode status window



Press RUN again. See Fig. 2 (next page) for typical values for Sample Voltage, etc. These values will fluctuate slightly while the machine runs but should be in this ballpark if all is running well. Note that Sample Voltage will be highest when the flow rate is Low and will decrease as you shift to Med and High.

Figure 2: Run mode status window